4-29-2020

Antimicrobial Activity of Extracts from the Leaves of Marrubium vulgare

Ashley Cravens
acravens@bellarmine.edu

Antimicrobial Activity of Extracts from the Leaves of *Marrubium vulgare*

Ashley Kay Cravens

Bellarmine University Honors Thesis

29 April 2020

Advisor: Dr. Amanda Krzysiak

Readers: Dr. Steven Wilt and Dr. Savita Chaurasia

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Abstract

Natural products have served as powerful therapeutics against pathogenic bacteria since the golden age of antibiotics of the mid-20th century. In the United States, antibiotics are a key component of modern medicine and are one of the top written prescriptions every year. However, overuse and misuse of antibiotics has led to an increasing frequency of antibiotic-resistant infections. These infections are difficult and expensive to treat, often resulting in increased length of illness, hospital stays, and mortality rates. This demonstrates a clear need for novel antibiotics in for modern medicine. *Murrubium vulgare* (white horehound) is a shrub found in the Appalachia region that has been used in herbal medicine to treat many pulmonary aliments as an expectorant or tonic. Previous studies of this plant and its commercial products have indicated that it may contain antioxidant and antibacterial properties. The aim of this study is to determine if whole leaf extracts of *M. vulgare* contain antimicrobial properties. Five different plants were sampled for this project. The leaves were dried and extracted in 95% ethanol in a 1:10 (m/v) ratio. The solvent was removed using a rotavapor at 60°C and hot plate evaporation. The crude product was left in a desiccator until constant weight was achieved. The crude samples were analyzed to characterize the different classes of natural products present. The crude extracts were dissolved in 5% DMSO at different concentrations. The disk diffusion assay was applied to determine the antimicrobial activity. The results indicated that *M. vulgare* did not have antimicrobial activity.

Background

Antibiotic Resistance

Overview

Antibiotics have played a crucial role in healthcare since the use of penicillin in the early 1940s. Since this time, nine main classes of antibiotics have been established and are currently used in healthcare.[1] There are nine different classes of antibiotics. β-lactams inhibit the synthesis of the cell wall. Tetracycline, aminoglycosides, lincosamides, and oxazolidinones inhibit protein synthesis. Fluoroquinolones and glycopeptides inhibit DNA synthesis. Sulfonamides inhibit RNA synthesis. Macrolides inhibits the synthesis of DNA or RNA.

Initially, antibiotics were described as any small molecule made from a microbe that antagonizes the growth of other microbes. This definition has changed to include synthetic molecules and larger organisms as sources of small molecules.[2] While microbe made antibiotics have diverse structures, they are assembled through enzyme catalyzed reactions using the same types of building blocks that closely resemble those used in making proteins, fatty acids, and polysaccharides. After the initial surge of antibiotic discovery, many pharmaceutical companies and researchers began to divert time and money into the development of treatments for other diseases. Current antibiotic research does not generally progress past the initial screening or design of a small molecule. This is thought to be a result of lack of antibacterial activity, misattribution of antibacterial activity, and a relatively high frequency of high-level resistance in bacteria.[3]

Bacteria can be placed into two categories when relating to a host organism: pathogenic and nonpathogenic. Pathogenic bacteria cause disease in a host, while nonpathogenic bacteria are harmless or useful to a host organism. The nonpathogenic bacteria can play an essential role in maintaining a homeostatic state within a host by preventing the contraction of pathogenic

bacteria and breakdown of nutrients is the gut. Antibiotics are selective in that they only affect bacteria and not the host, but they do not discriminate between the types of bacteria living in the host. This can be problematic as important, nonpathogenic bacteria are destroyed when taking antibiotics.[2] Typically, antibiotics work by preventing a crucial step in the cell cycle of the bacteria cell.[4]

Antibiotics are one of the most commonly prescribed drugs in the United States, with nearly 50 percent of these being prescribed unnecessarily. From 2000 to 2010, these was an estimated 1.4 billion outpatient antibiotics dispensed in the United States. During this time antibiotic prescription in general remained steady; however, there was a decrease in the antibiotic prescriptions to young children and an increase in antibiotic prescription for adults over the age of 65.[5] In 2016, there was an estimated 270 million antibiotic prescriptions for both inpatients and outpatients.[6]

Rise of Antibiotic Resistance

The misuse and overuse of antibiotics has led to what is known as antibiotic resistant bacteria. Bacteria that are resistant to antibiotics are able to breakdown the antibiotic molecule or prevent its uptake into the cell.[7] Antibiotic resistance arises due to random mutation in bacterial genes and has an increased prevalence due horizontal gene transfer and "survival of the fittest." In horizontal gene transfer, bacteria are able to transmit plasmids (DNA) to one another. If a plasmid contains a gene that averts an antibiotic, then the bacteria that contain the plasmid will be resistant to that antibiotic. In the "survival of the fittest," the non-resistant bacteria die in the presence of an antibiotic, leaving more room and nutrients for the growth and replication of resistant bacteria.

Antibiotic resistance infections can be acquired through two methods: contraction of antibiotic resistant bacteria from an outside source or antibiotic resistance occurring due to the use of antibiotics. Resistant bacterial infections have personal, economic, and social repercussions. On a personal level, antibiotic resistance leads to longer illness and increased hospital stays.[2] The need for an increased level of care and use of more expensive, second-line treatment options increases the cost of healthcare, which has an economic impact.[8] One study has shown that antibiotic resistant infection in the United States cost the healthcare system more than 20 billion dollars annually.[2] Socially, there is an increased mortality and a greater risk to individuals who have compromised immune systems, such as those undergoing major surgeries and chemotherapy. As of 2013, there have been at least two million illnesses and 23,000 deaths that have been contributed to antibiotic resistant bacteria in the United States.[1]

Proposed Solutions

In response to the rise in antibiotic resistant bacteria, the World Health Organization (WHO) outlined a five-step plan to negate the rising severity of the situation. (Table 1) [9] The goal of my research is to contribute to the investment in countering antimicrobial resistance by exploring natural products as a source of new antibacterial drug discovery. I will be studying the leaves of *M. vulgare* as a novel source of antimicrobial activity. By exploring new natural products, the scientific community gains information on new potential sources of antibiotics. It is essential to continuing investigating new potential sources of antibiotics, due to the rise in antibiotic resistance which is limiting the effectiveness of current antibiotics.

Table 1: World Health Organization objectives and initiatives

Objective	Initiatives
To improve awareness and understanding of antimicrobial resistance.	World Antibiotic Awareness Week
To strengthen surveillance and research.	The Global Antimicrobial Resistance Surveillance System
To reduce the incidence of infection.	Global Antibiotic Research and Development Partnership
To optimize the use of antimicrobial medicines.	Interagency Coordination Group on Antimicrobial Resistance
To ensure sustainable investment in countering antimicrobial resistance.	Global Antibiotic Research and Development Partnership

This table outlines the five objectives that the World Health Organization (WHO) has set to battle rising antibiotic resistance. To address the objectives, WHO established initiatives that individuals, organizations, and countries can contribute too.

Natural Products

Overview

Natural products have historically shown an exceedingly good track record when it comes to the development of new antibiotics. Penicillin, the first antibiotic, was derived from a mold secondary metabolite. Currently, six of the nine classes of antibiotics are natural product derivatives.[1] Secondary metabolites are organic compounds produced by bacteria, fungi, or plants that are not directly involved in normal growth, development, or reproduction of the organism.[1] These compounds can exhibit many biological activities, including antimicrobial, antifungal, anticancer, and anti-inflammatory activities.

Different Classes of Natural Products

Natural products can be divided into many different categories based on their chemical structure, properties, etc. There are five different categories based on their biosynthetic pathway and carbon skeletal structure: (1) peptides and proteins, (2) polyketides and fatty acids, (3) terpenoids and steroids, (4) alkaloids, and (5) phenylpropanoids. Each of these categories of

natural products derive from primary metabolic biosynthetic pathways (Figure 1).[10, 11] The scope of this review will only include secondary products that have shown potential antibacterial properties: primarily alkaloids, flavonoids, tannins, terpenes, quinones, and resins. (Table 2)

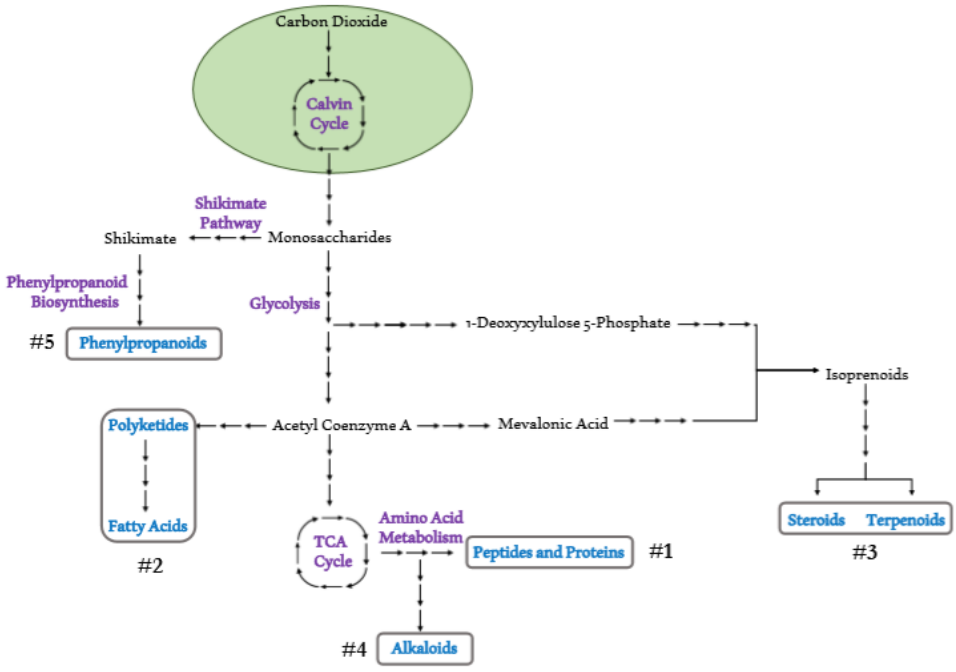

Figure 1. Pathway of secondary metabolite biosynthesis. The pathways that result in the five main chemical categories of natural products. Taken from Stantliff.[12]

Table 2. Secondary metabolites from different plant families and their reported antibacterial activities

Table 1: Secondary metabolites from different plant families and their reported antibacterial activities

Secondary metabolite and plant families	Antibacterial activity	References
Alkaloids		
Fabaceae	*Escherichia coli*	Carson and Hammer (2010)
Amaryllidaceae	*Staphylococcus aureus*	Savoia (2012)
Mimosaceae	*Pseudomonas aeruginosa*	Ramawat (2007)
Capparaceae	*Mycobacterium tuberculosis*	Agbafor *et al.* (2011)
Rubiaceae	*Mycobacterium kansaii*	Mariita *et al.* (2011)
Compositae	*Salmonella typhi*	Munyendo *et al.* (2011)
Rutaceae	*Staphylococcus epidermidis*	Sibi *et al.* (2012)
	Methicillin-resistant *Staphylococcus aureus* (MRSA)	
Flavonoids		
Fabaceae	*Pseudomonas aeruginosa*	Agbafor *et al.* (2011)
Amaryllidaceae	*Mycobacterium fortuitum*	Munyendo *et al.* (2011)
Rubiaceae	*Staphylococcus aureus*	Sibi *et al.* (2012)
Labiatae	*Salmonella typhi*	
Rutaceae	*Staphylococcus epidermidis*	
	Methicillin-resistant *Staphylococcus aureus* (MRSA)	
Tannins		
Myrtaceae	*Staphylococcus aureus*	Abdulhamid *et al.* (2014)
Fabaceae	*Streptococcus faecalis*	Mariita *et al.* (2011)
Mimosaceae	*Bacillus subtilis*	Sibi *et al.* (2012)
Rubiaceae	*Escherichia coli*	Oboh (2010)
Labiatae	*Salmonella typhi*	
	Pseudomonas aeruginosa	
	Staphylococcus epidermidis	
	Shiegella dysentria	
	Proteus sp.	
Terpenes		
Myrtaceae	*Streptococcus faecalis*	Sibi *et al.* (2012)
Rubiaceae	*Pseudomonas aeruginosa*	Abdulhamid *et al.* (2014)
Compositae	*Staphylococcus epidermidis*	Munyendo *et al.* (2011)
Labiatae	Methicillin-resistant	Korir *et al.* (2012)
Rutaceae	*Staphylococcus aureus* (MRSA)	
Caesalpinaceae		
Amaranthaceae		
Quinones		
Boraginaceae	*Staphylococcus aureus*	Papageorgiou *et al.* (2008)
Plumbaginaceae	*Staphylococcus epidermidis*	Carson and Hammer (2010)
Ebanaceae		Savoia (2012)
Droseraceae		Ramawat (2007)
Resins		
Labiatae	*Pseudomonas aeruginosa*	Oboh (2010)
Fabaceae	*Shiegella dysentria*	Mariita *et al.* (2011)
	Proteus sp.	
	Staphylococcus aureus	
	Mycobacterium fortuitum	
	Salmonella typhi	

Taken from Crozier.[13]

Alkaloids are organic heterocyclic nitrogen compounds that are basic-forming water-soluble salts (Figure 2).[8] These compounds are mostly derived from amino acids and are found in about 20 percent of plant species. Alkaloids are through to play a defensive role in the plant against herbivores and pathogens. Well known alkaloids of plant origin include caffeine, nicotine, and morphine.[13]

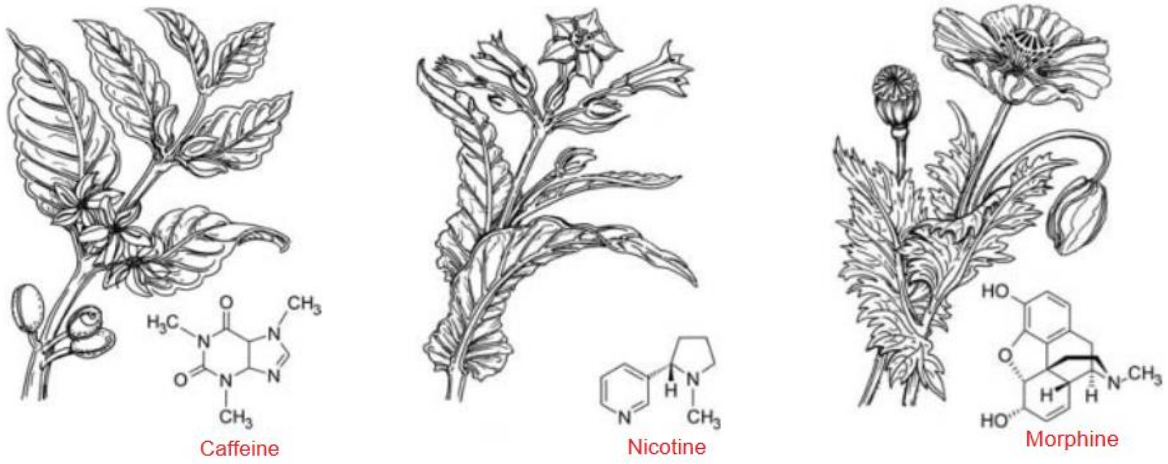

Figure 2. Structures of common alkaloids. *Coffea arabica* and caffeine. *Nicotiana tabacum* and nicotine. *Papaver somniferum* and morphine. Taken from Crozier et al.[13]

Flavonoids are polyphenolic structures that contain a flavine nucleus (2-phenyl-benzopyrane), which consists of two benzene rings linked through a heterocyclic pyrane ring (Figure 3). There are currently 14 classes of flavonoids identified that differ based on chemical nature and position of substituents on the different rings.[8] These are present in high concentrations in the epidermis of leaves and the skin of fruits. Flavonoids play important in UV protection, pigmentation, stimulation of nitrogen-fixing nodules, and disease resistance.[13]

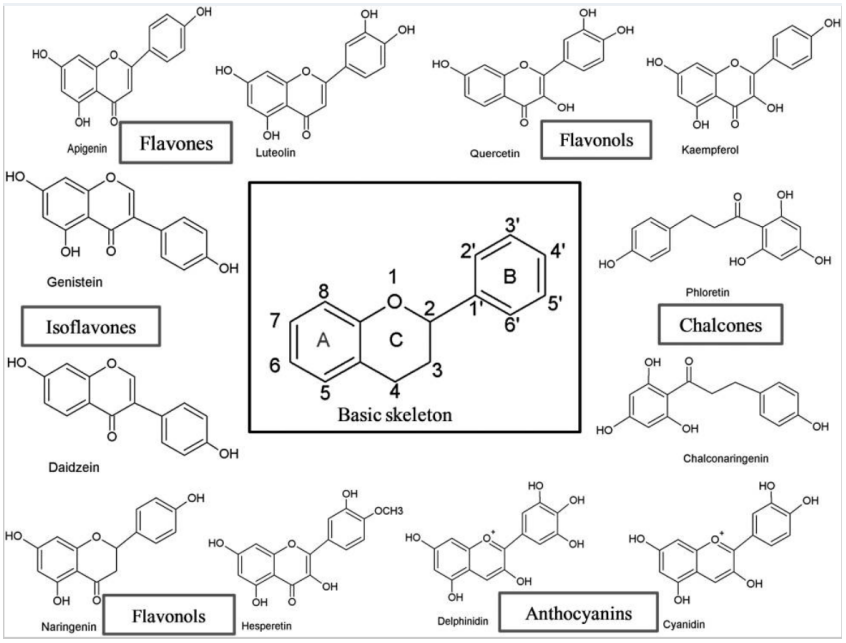

Figure 3. Structure of flavonoids and common classes. Taken from Panche et al.[14]

Tannins are polymeric phenolic substances and can be found in nearly all plant parts (Figure 4). These have been known to display a variety of biological activities including antifungal and antibacterial.[8]

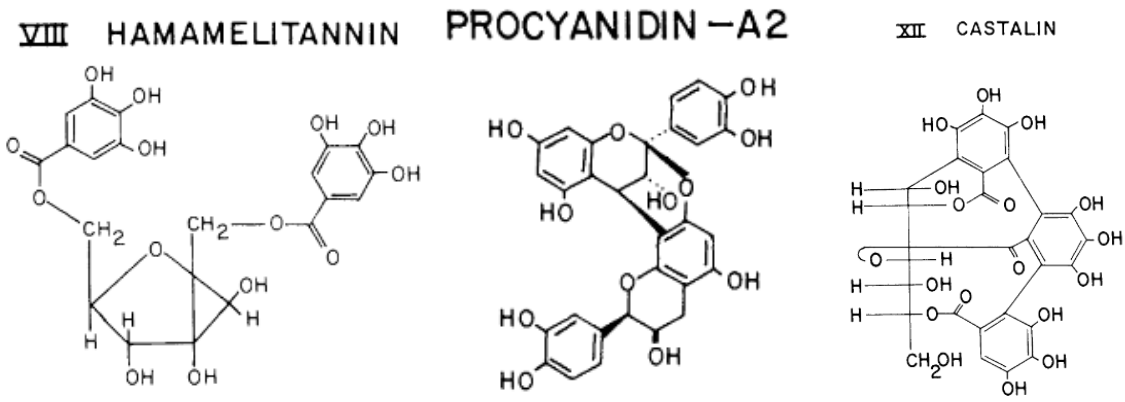

Figure 4: structure of common tannins. Hamamelitannin (Gallotannin), Condensed Tannin (Procyanidin), Ellagitannin (Castalin). Taken from Zucker.[15]

Terpenes are one of the most diverse classes of metabolites with over 30,000 varieties and are identifiable by the number of isoprene units, which consists of a branched-chain

unsaturated hydrocarbon.[8] These are used in flavoring, fragrances, antibiotics, plant and animal hormones, and mediators of essential electron-transport processes.[13] A commonly known terpene is cholesterol (Figure 5).

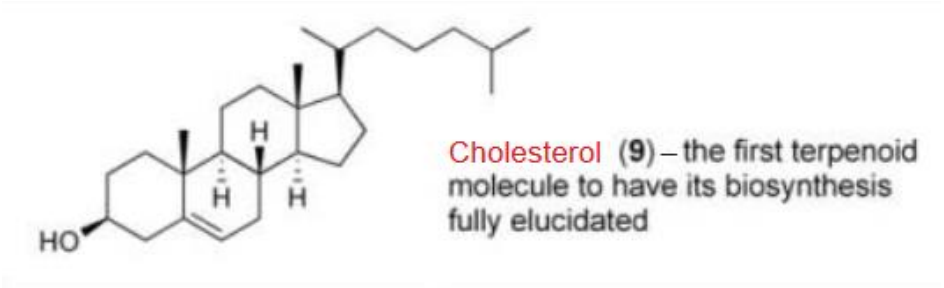

Figure 5. Structure of cholesterol. Taken from Crozier.[13]

Quinones are compounds that contain aromatic rings with two ketone substitutions (Figure 6). There have been over 400 naturally occurring quinones, which are found in all plant organs.[8]

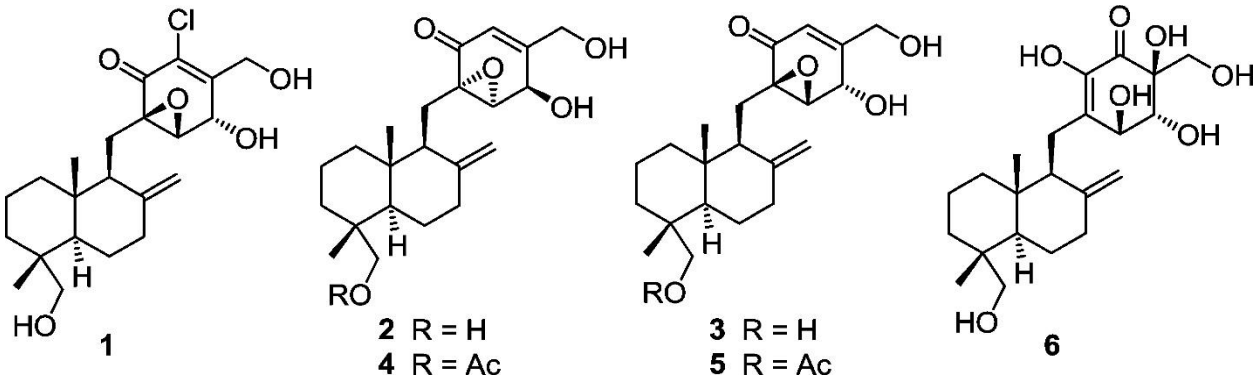

Figure 6. Structure of quinones from isolated cultures of *Myrothecium* sp. Taken from Fu.et al.[16]

Resins are non-volatile plant compounds that are found in plants as mixtures of compounds, such as flavonoids, terpenoids, fatty substances, etc. (Figure 7).[8]

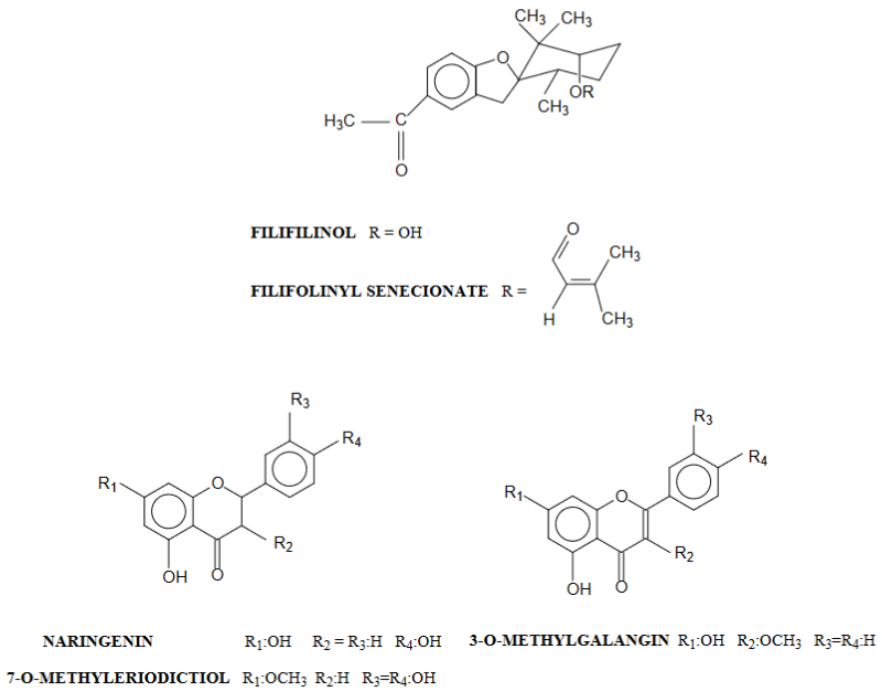

Figure 7. Structure of isolated compounds from exudates resins of *Heliotropium taltalense*. Taken from Modak et al.[17]

Marrubium vulgare

Marrubium vulgare, also known as the white horehound, is a shrub that grows in poor nutrient environments. This plant is native to the Appalachia region and is traditionally used in pulmonary ailments as an expectorant, tonic, emmenagogue. The leaves were boiled and used to make cough syrup.[18] I have chosen to work with this plant due to its low maintenance, previous use in medicine, and prior research indicating potential antimicrobial properties.[19-21]

Review of Previous Research Concerning *Marrubium vulgare*

"The In-Vitro Evaluation of Antibacterial, Antifungal and Cytotoxic Properties of Marrubium vulgare L. Essential Oil Grown in Tunisia"

The goal of this study was to determine if *M. vulgare* essential oil contained any antibacterial, antifungal, or cytotoxic activity. Agar disk diffusion was used to test the antibacterial and antifungal properties of twelve bacterial strains and four fungi strains. The bacterial assay showed some antimicrobial activity for gram positive bacteria, while there was no

antimicrobial activity for gram negative bacteria. There was antifungal activity for all four types of fungi. However, the results are inconclusive due to the potential presence of synergy, antagonism, or additive effects of the tested major components of the oils, which possess various potency of activity. An MMT assay using HeLa cells was used to determine the cytotoxic properties of the essential oil. The results of the cytotoxic assay showed a significant decrease in the viability of the HeLa cells. These results are promising with regards to possible antineoplastic chemotherapies in the future.[19] This study is useful to my research since it indicates that *M. vulgare* may be more effective against gram positive bacteria.

"Marrubium vulgare L. Leave Extract: Phytochemical Composition, Antioxidant and Wound Healing Properties"

This study aimed to explore *M. vulgare* leaf extracts as a potential wound healing agent. Leaf extracts were obtained using a 80/20 methanol/water extraction on air-dried leaves. The extraction was screened to determine the presence of plant secondary metabolites. These extracts contained high concentration of flavonoids and phenols. These extracts were tested for antioxidant activity through a DPPH assay. The fibroblast proliferation was determined using an MTT assay using a human dermal fibroblast cell line. These tests indicate antioxidant activity and high proliferation and migration of fibroblast cell line. These tests indicated antioxidant activity and high proliferation and migration of fibroblast, respectively.[20] This study indicates which potential types of secondary metabolites that might be recovered. It also outlines an extraction method that has been successful when working with *M. vulgare*. This study does not analyze the potential antimicrobial activity of the extracts, leaving then as an unexplored avenue of research.

"Antibacterial Activity of Whole Plant Extract of Marrubium vulgare"

The objective of this study was to determine the antibacterial activity of a whole plant extract of *M. vulgare.* A *M. vulgare* plant was prepared for extraction by leaving it in the sun to dry then grinding it into a fine powder. 200 grams of the powder underwent extraction using five different concentrations of methanol. The antibacterial activity was tested against six different strains of bacteria using the disc diffusion method. The extracts has moderate to significant results for five of the six bacteria strains.[21] This study indicates that *M. vulgare* has the potential for antibacterial properties. I will expand upon this research by focusing a singular part of the plant as well as determine the secondary metabolites within the plant.

Materials and Methods

Plant Material

M. vulgare were purchased from The Growers Exchange (Sandston, VA) and planted at a local farm. Plants were grown in half shade conditions (Figure 8). The plants were allowed to grow between five and six months before harvesting.

Figure 8. *M. vulgare* Garden

Harvesting and Preparation of Plant Material

Fresh samples of *M. vulgare* leaves were collected in the lab. These leaves were rinsed with water and dried in food evaporator at 30°C for 48 hours. Dried material was sealed in mason jars at room temperature until further use.

Maceration of Crude Extracts

Dried material was placed in an Erlenmeyer flask. 95% ethanol was added in a 1:10 (m/v) ratio. These flask was shaken for 48 hours at room temperature.[22]

Filtration and Solvent Evaporation

After the maceration period, the leaf-solvent mixtures were filtered through a Whatman No. 1 filer paper. This solution was concentrated to approximately 12mL with a rotary evaporator at 60˚C. This was transferred quantitatively to a pestle using 95% ethanol and heated on a hot plate until remaining solvent was evaporated.[22]

Qualitative Assay of Phytochemicals

Tannins

A small amount of plant material was dissolved in 2mL of 95% ethanol. Then 2 to 3 drops of 10% ferric chloride was added to the solution. A resulting blue-green or blue-black coloration indicates the presence of tannins.[23]

Phlobatannis

A small amount of plant material was dissolved in 0.5mL of 95% ethanol. Then 0.5ml of the solution was added to 0.5mL of 1% aqueous hydrochloric acid. This solution was then brought to a boil. The deposition of a red precipitate is indicative of the presents of phlobatannins.[23]

Phenols

A small amount of plant material was dissolved in 0.5mL of 95% ethanol. Then 2 to 3 drops of 10% aqueous ferric chloride was added to the solution. The appearance of blue or green coloration indicates the presence of phenols.[23]

Flavonoids

A small amount of plant material was dissolved in 0.5mL of 95% ethanol. Then 2 to 3 drops of 20% sodium chloride solution was added to the solution. The appearance of an intense yellow color that turns colorless upon the addition on a few drops of dilute acid, is indicative of the presence of flavonoids.[23]

Alkaloids

A small amount of plant material was dissolved in 0.5mL of 95% ethanol. Then 2mL of Wagner's reagent was added to the solution. The formation of a reddish/brown precipitate indicates the presence of alkaloids.[23]

Steroids

A small amount of plant material was dissolved in 0.5mL of 95% ethanol. Then 2mL of chloroform and a small amount of concentrated H_2SO_4 was added to the solution. The appearance of a red color in the chloroform layer indicates the presence of steroids.

Free Anthraquinones

A small amount of plant material was dissolved in 0.5mL of 95% ethanol. Then 1mL of chloroform was added to the solution and was shake for 5 minutes. The solution was then filtered using Whatman No. 1 filer paper. An equal volume of 10% ammonia solution was added to the filtrate and shaken. The appearance of pink, red, or violet color in the aqueous layer indicates the presence of free anthraquinones.

Test Microorganisms

In vitro antimicrobial studies were carried out on two Gram-positive bacteria (*Bacillus subtillis*, *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia Coli. Proteus vulgaris*), which were purchased from Carolina Biological Supply Company (Burlington, NC). The cultures were reactivated according to manufacturer instructions. *S. aureus, E. Coli,* and *P. vulgaris* were allowed to grow in a 37°C incubator for 24 hours. These were then streaked on nutrient agar plates, allowed to grow in a 37°C incubator for 24 hours, and subsequently place the 4°C refrigerator until needed. The same procedure was performed for *B. subtilis*, except it was placed in the 30°C incubator.

Culture Media

Nutrient Broth

Nutrient broth was purchased from Carolina Biological Supply Company (Burlington, NC). 8.0 grams of the media was mixed in one liter of purified water until evenly dispersed. The mixture then underwent heating and stirring until the media was completely dissolved. It was then autoclaved at 121°C for 15 minutes.

Nutrient Agar

Nutrient agar was purchased from Carolina Biological Supply Company (Burlington, NC). 23.0 grams of the media was mixed in one liter of purified water until evenly dispersed. The mixture then underwent heating and stirring until the media was completely dissolved. It was then autoclaved at 121°C for 15 minutes.

Antimicrobial Susceptibility Test

Disk Diffusion Method

8mL of nutrient agar broth was added to 4 culture vials, respectivly. *S. aureus, E. Coli, B. subtilis* and *P. vulgaris* were removed from stock plates in 4°C refrigerator using a sterile inoculating loop and subsequently added to individual culture vials. These were placed in 37°C shaking incubator overnight. 800mg of plant materal was dissolved into 1mL of 5% DMSO through heating and shaking. It was subsequently diluted to the following concentrations: 600mg/ml, 400mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml. 15uL of the desired plant sample and 5% DMSO was loaded onto respective 6mm Whatmann AA Grade Discs on the day of testing. 5ug Ciprofloxacin Sensi Disc were ordered from Becton, Dickinson, and Company (Sparks, MD) and used as a postivive control. Using the spectrometer set at 600nm, the bacteria samples were diluted to an absorbance of 0.4A. Nutrient agar plates were divied into four quadrants and inoculated with 500uL of the appropritate diluted bacteria stock.The inoculated

filter paper and positive control was placed in the center of each quadrant. The plates were placed in 30°C or 37°C incubator, depending on the preference of the bacteria samples. The zone of inhibition was measured after 24 hours.[24]

Results

Crude Extract Yield

The percent yield was calculated using the initial dry weight of *M. vulgare* used in the extraction process and the final weight of the extraction.

$$Percent\ Yield = \frac{Extract\ Weight}{Initial\ Plant\ Weight}$$

Table 3. Summary of Percent Yield

Plant	Extract Weight (g)	Initial Plant Weight (g)	Percent Yield
1	2.9	55.6	0.052
2	2.8	54.9	0.051
3	3.8	64.1	0.059
4	4.8	73.8	0.065
5	3.1	44.2	0.070

Qualitative Assay of Phytochemicals

Each of the plants used in this experiment were screened for a variety of phytochemicals, including tannins, phlobatannis, phenols, flavonoids, alkaloids, steroids, and free anthraquinones. All of the plants screened identically for each test and the results are summarized in Table 4. Phytochemical screening indicated the presence of tannins, phlobatannis, phenols, and steroids in the leaves of *M. vulgare.* These chemicals have shown antibacterial activity in other sources of natural products.

Table 4. Summary of Phytochemical Screening

Plant	Tannins	Phlobatannis	Phenols	Flavonoids	Alkaloids	Steroids	Free Anthraquinones
1	Positive	Positive	Positive	Negative	Negative	Positive	Negative
2	Positive	Positive	Positive	Negative	Negative	Positive	Negative
3	Positive	Positive	Positive	Negative	Negative	Positive	Negative
4	Positive	Positive	Positive	Negative	Negative	Positive	Negative
5	Positive	Positive	Positive	Negative	Negative	Positive	Negative

Disc Diffusion Assay

Each of the dried plant extracts were dissolved in 5% DMSO. Then dilution was preformed to create 800mg/uL, 600mg/uL, 400mg/uL, 200mg/uL, 100mg/uL, and 50mg/uL samples. These were plated on four different bacteria samples as well as 5ug ciprofloxacin (positive control) and 5% DMSO (negative control). All of the plant samples and the 5% DMSO did not create a zone of inhibition, while the ciprofloxacin created variously sized zones depending on the bacteria (Table 5). The plant extracts did not create a zone of inhibition, indicating that these did not possess antimicrobial activity. The ciprofloxacin did create zones of inhibition indicating the bacteria was susceptible to effective antibacterial sources. To illustrate the results of the disc diffusion assay, I choose to include the bacterial plates from the Plant 5 assays (Figures 9-12).

Table 5. Zone of Inhibition for Ciprofloxacin

	Plant 1 (Average Zone of Inhibition, mm)	Plant 2 (Average Zone of Inhibition, mm)	Plant 3 (Average Zone of Inhibition, mm)	Plant 4 (Average Zone of Inhibition, mm)	Plant 5 (Average Zone of Inhibition, mm)	Average (mm)
B. subtilis	9.67	12.33	10.33	10.33	9.67	10.47
E. coli	14.33	12	15.33	13.67	14.33	13.93
S. aureus	13.33	10.33	13.33	11.67	13.67	12.47
P. vulgaris	10.67	10.67	9	9.67	10	10

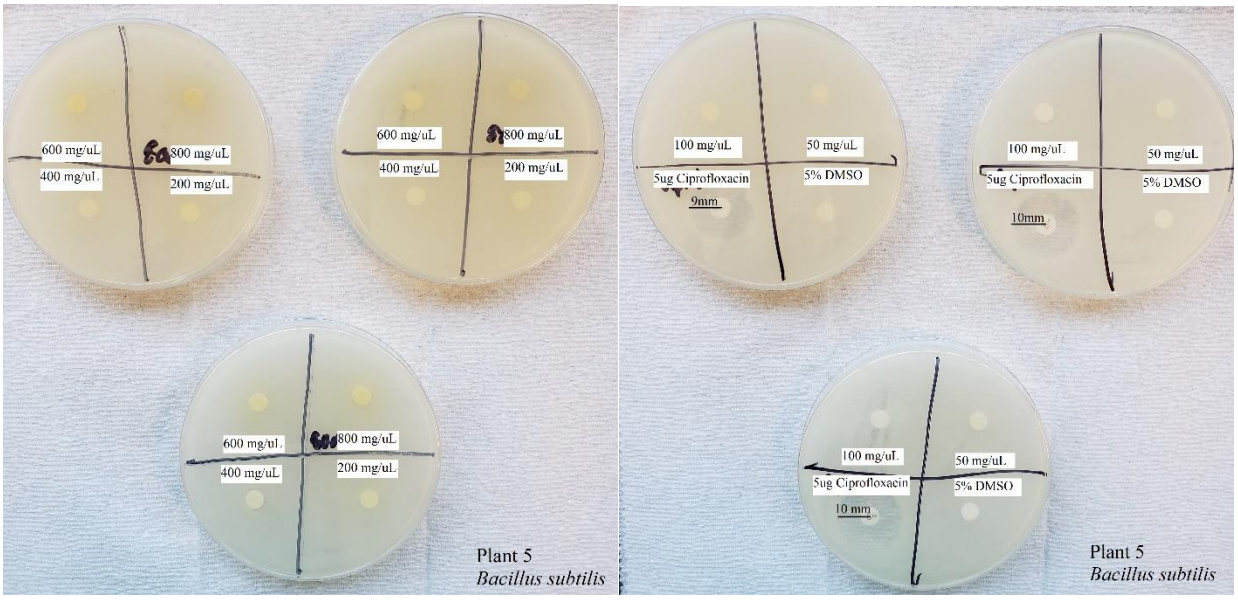

Figure 9. Plant 5 – *Bacillus subtilis*

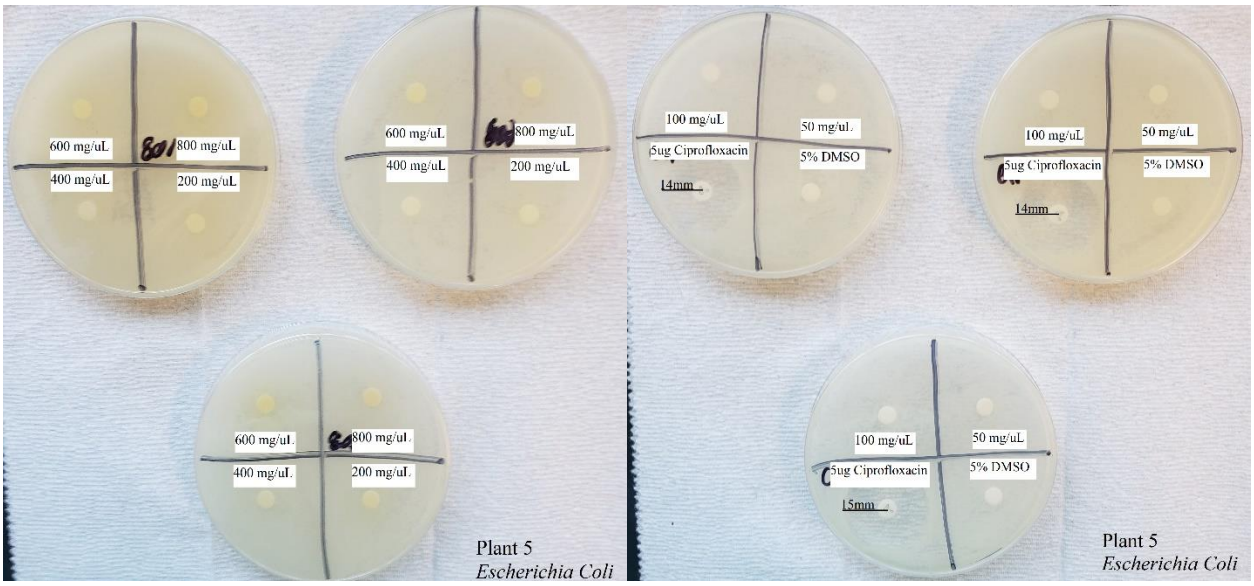

Figure 10. Plant 5 – *Escherichia Coli*

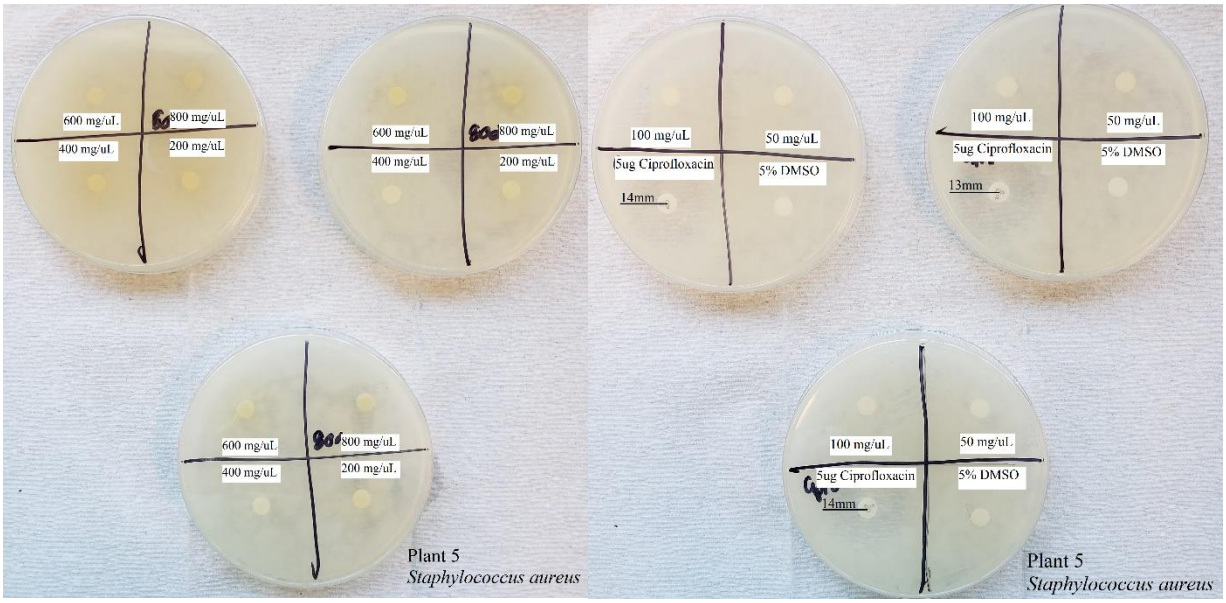

Figure 11. Plant 5 – *Staphylococcus aureus*

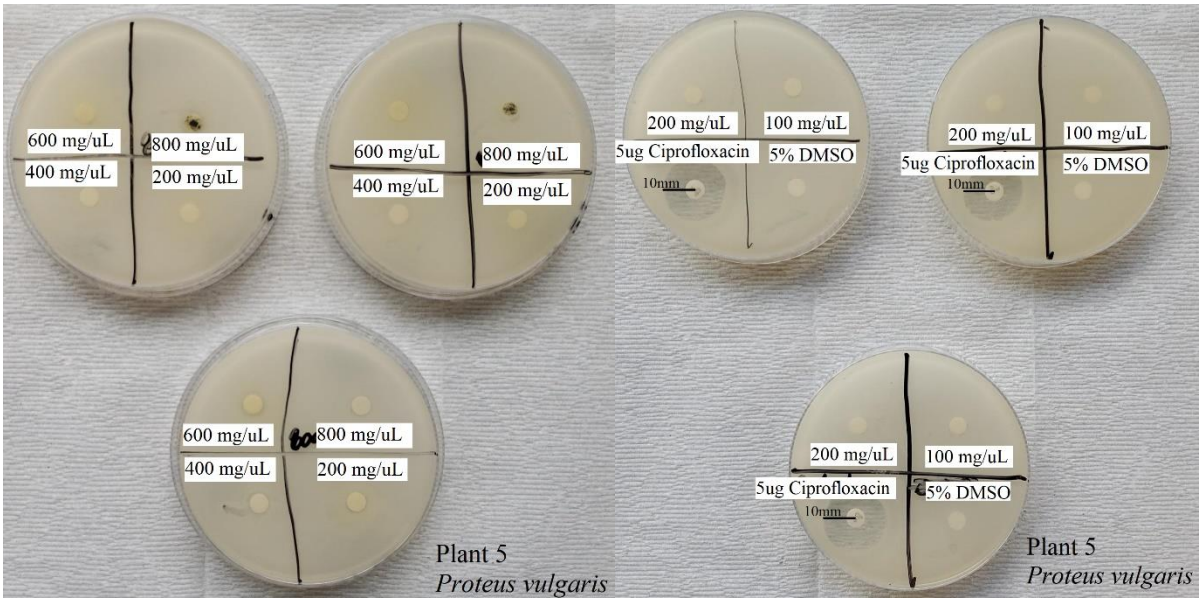

Figure 12. Plant 5 – *Proteus vulgaris*

Discussion

The purpose of this project was to evaluate the antimicrobial activity of the leaves of *M. vulgare*. The phytochemical analysis indicated the presence of tannins, phlobatannis, phenols, and steroids. While these chemicals have shown antimicrobial properties in other organisms, the leaves of this plant did not show antimicrobial activity during the disc diffusion analysis. The lack of antimicrobial activity is evident due to the failure of the samples to produce a zone of inhibition. The positive control, ciprofloxacin 5ug, did create zones of inhibition on all bacterial plate. Although the leaves of this particular plant did not indicate antimicrobial activity, previous studies of the entire plant did show antimicrobial activity.[21] This suggests that further analysis of the roots and flowers of this plant are necessary to isolate the location of the molecules responsible for this activity. Furthermore, a previous study of the essential oil of *M. vulgare* indicated the presence of antioxidant activity.[20] Flavonids, tannins, and phenols, all of which were found in the leaf extracts, have been shown to possess antioxidant activity in different plant species. Antioxidants are molecules with a radical-scavenging capacity that are thought to exert a protective effect against free radical damage. These biomolecules may contribute to the prevention of many chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, diabetes, asthma, hepatitis and arthritis.[25] Further analysis of my plant extracts is necessary to determine their antioxidant activity. Finally, it is important to reiterate the continual rise in antimicrobial resistant bacteria. As of 2013, there have been at least two million illnesses and 23,000 deaths that have been contributed to antibiotic resistant bacteria in the United States. This will continue to be a growing issue as more antibiotics become ineffective in treatment. Due to

historical success of natural products in the development of new source of antibiotics, continual research of natural products is recommended to develop new antibiotics.

References

1. Rossiter, S. E.; Fletcher, M. H.; Wuest, W. M., Natural Products as Platforms To Overcome Antibiotic Resistance. *Chemical reviews* **2017,** *117* (19), 12415-12474.
2. Emanuele, P., Antibiotic Resistance. *AAOHN Journal* **2010,** *58* (9), 363-365.
3. Gualerzi, C. O.; Brandi, L.; Fabbretti, A.; Pon, C. L., *Antibiotics : Targets, Mechanisms and Resistance*. John Wiley & Sons, Incorporated: Weinheim, GERMANY, 2013.
4. Clardy, J.; Fischbach, M. A.; Currie, C. R., The natural history of antibiotics. *Current Biology* **2009,** *19* (11), R437-R441.
5. Lee, G. C.; Reveles, K. R.; Attridge, R. T.; Lawson, K. A.; Mansi, I. A.; Lewis, J. S., 2nd; Frei, C. R., Outpatient antibiotic prescribing in the United States: 2000 to 2010. *BMC medicine* **2014,** *12*, 96.
6. *Outpatient antibiotic prescriptions*. *Centers for Disease Control and Prevention*: United States, 2016.
7. Antibiotic Resistance. World Health Organization: 2018; Vol. 2019.
8. Compean, K. L.; Ynalvez, R. A., Antimicrobial Activity of Plant Secondary Metabolites: A Review. *Research Journal of Medicinal Plant* **2014,** *8* (5), 204-213.
9. Newman, D. J.; Cragg, G. M., Natural Products as Sources of New Drugs from 1981 to 2014. *Journal of Natural Products* **2016,** *79* (3), 629-661.
10. Williams, D. H.; Stone, M. J.; Hauck, P. R.; Rahman, S. K., Why are secondary metabolites (natural products) biosynthesized? *Journal of natural products* **1989,** *52* (6), 1189-208.
11. Hanson, J. R., *Natural products : the secondary metabolites*. Royal Society of Chemistry: Cambridge, 2003.
12. Stantliff, T., Investigations of the Mechanism of Action for Lung Cancer Cell Death by a 4-Trifluoromethoxy Substituted Chalcone. Bellarmine University: 2018.
13. Crozier, A.; Clifford, M. N.; Ashihara, H., Plant Secondary Metabolites : Occurrence, Structure and Role in the Human Diet. John Wiley & Sons: Chichester, 2007. http://www.123library.org/book_details/?id=25352http://www.myilibrary.com?id=132015https://public.ebookcentral.proquest.com/choice/publicfullrecord.aspx?p=351346http://www.myilibrary.com?id=132015&ref=toc.
14. Panche, A. N.; Diwan, A. D.; Chandra, S. R., Flavonoids: an overview. *Journal of nutritional science* **2016,** *5*, e47-e47.
15. Zucker, W. V., Tannins: Does Structure Determine Function? An Ecological Perspective. *The American Naturalist* **1983,** *121* (3), 335-365.
16. Fu, Y.; Wu, P.; Xue, J.; Wei, X., Cytotoxic and Antibacterial Quinone Sesquiterpenes from a Myrothecium Fungus. *Journal of natural products* **2014,** *77* (8), 1791-9.
17. Modak, B.; Rojas, M.; Torres, R., Chemical analysis of the resinous exudate isolated from Heliotropium taltalense and evaluation of the antioxidant activity of the phenolics components and the resin in homogeneous and heterogeneous systems. *Molecules (Basel, Switzerland)* **2009,** *14* (6), 1980-9.
18. Bhavnani, S. M.; Ballow, C. H., New agents for Gram-positive bacteria. *Current Opinion in Microbiology* **2000,** *3* (5), 528-534.

19. Zarai, Z.; Kadri, A.; Ben Chobba, I.; Ben Mansour, R.; Bekir, A.; Mejdoub, H.; Gharsallah, N., The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of Marrubium vulgare L. essential oil grown in Tunisia. *Lipids in health and disease* **2011,** *10*, 161-161.
20. Amri, B.; Martino, E.; Vitulo, F.; Corana, F.; Ben-Kaâb, L. B.; Rui, M.; Rossi, D.; Mori, M.; Rossi, S.; Collina, S., Marrubium vulgare L. Leave Extract: Phytochemical Composition, Antioxidant and Wound Healing Properties. *Molecules* **2017,** *22* (11), 1851.
21. *Masoodi, M.*; *Ahmed, B.*; *Zargar, I.*; *Khan, S.*; *Khan, S.*; *Singh, P.*, Antibacterial activity of whole plant extract of *Marrubium vulgare*. *African Journal of Biotechnology* **2008,** *7* (2), 086-087.
22. Yeo, Y.; Chia, Y.; Lee, C. H.; Sow, H.; Yap, W.-S., Effectiveness of Maceration Periods with Different Extraction Solvents on in-vitro Antimicrobial Activity from Fruit of Momordica charantia L. *Journal of Applie Pharmaceutical Science* **2016,** *4*, 016-023.
23. Auwal, M. S.; Saka, S.; Mairiga, I. A.; Sanda, K. A.; Shuaibu, A.; Ibrahim, A., Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). *Veterinary research forum : an international quarterly journal* **2014,** *5* (2), 95-100.
24. Bayot, M., Antimicrobial Susceptibility Testing. Bragg, B., Ed. StatPearls: 2018.
25. Formagio, A. S. N.; Volobuff, C. R. F.; Santiago, M.; Cardoso, C. A. L.; Vieira, M. d. C.; Valdevina Pereira, Z., Evaluation of Antioxidant Activity, Total Flavonoids, Tannins and Phenolic Compounds in Psychotria Leaf Extracts. *Antioxidants (Basel, Switzerland)* **2014,** *3* (4), 745-757.